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ORIGINAL ARTICLE

Fish pollutants MeHg and Aroclor cause permanent structural damage in male gonads and kidneys after prepubertal exposure

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SUMMARY

This study investigated whether or not prepubertal exposure to the fish contaminants methylmercury (MeHg) and the polychlorinated bisphenol Aroclor in low doses interferes with the histomorphometry of the testes, epididymis, liver and kidneys in rats. Wistar male rats, 21 days old, were allocated into the following: control (n = 17, received corn oil), MeHg (n = 17, received MeHg at 0.5 mg/kg/day), Aroclor (n = 17, received Aroclor at 1.0 mg/kg/day), low mix (n = 18, received MeHg at 0.05 mg/kg/day and Aroclor at 0.1 mg/kg/day), high mix (n = 18, received MeHg at 0.5 mg/kg/day and Aroclor at 1.0 mg/kg/day). Dosing continued from post natal day (PND) 23 to 53, by gavage. Euthanasia was performed on PND 53; or, after an interval of 62 days without exposure to chemicals, on PND 115. The degree of maturation of the seminiferous epithelium was delayed in chemical-exposed groups and testicular interstitial oedema was observed at adulthood. The pattern of male gonad organization was changed in the Aroclor group on PND 53 and in all treated groups at adulthood. The animals from Aroclor, low mix and high mix groups showed a reduction in the number of Sertoli cells. Histological evidence of renal injury was observed in all chemical-exposed groups in both ages. A probable target for MeHg and Aroclor in the reproductive system was Sertoli cells, in which possible dysfunctions could be linked to the other testicular alterations. Curiously, the main deleterious effects were late outcomes, along with the absence of synergistic interaction of MeHg and Aroclor in the parameters investigated. In conclusion, fish pollutants MeHg and Aroclor caused permanent structural damage in male gonads and kidneys after prepubertal exposure, without showing clear chemical interactions.

Keywords
chemical mixtures, fish contaminants, methylmercury, peripuberty, polychlorinated bisphenols, rats

In recent decades continuous exposure of human population to complex mixtures of toxic substances has been of increasing interest to the scientific community. Among these compounds, methylmercury (MeHg) and polychlorinated bisphenols (PCBs) deserve special attention, as chronic exposure to low doses of these chemicals occurs in different parts of the world (Roda *et al.* 2012). MeHg and PCBs are persistent toxic agents in the environment and undergo biomagnification along the trophic chain. The main source of human exposure is the consumption of fish and other contaminated seafoods (Mahaffey *et al.* 2009; Chen 2012; Oken *et al.* 2012; Sunderland & Selin 2013). Since MeHg and PCBs often occur together in the environment, the possible ways of interaction between them, such as additive, synergistic or antagonistic effects, needs to be thoroughly investigated (Cory-Slechta 2005). *In vitro* (Bemis & Seegal 1999, 2000, 2004) and *in vivo* (Roegge *et al.* 2004; Roegge & Schantz 2006) studies have suggested a synergistic effect. However, these data are controversial (Widholm *et al.* 2004; Vitalone *et al.* 2010; Coccini *et al.* 2011).

Some countries have published guidelines on fish consumption aiming to minimize public exposure to contaminants, particularly MeHg. In 2004, the FDA (United States Food and Drug Administration) and the EPA (United States Environmental Protection Agency) recommended that pregnant or lactating women, young children and women who were planning a pregnancy should not consume some fish species, which had a higher degree of contamination by MeHg (Lando *et al.* 2012). However, these initiatives generally have had little impact on the eating habits of the population. While regulatory agencies have recommended restrictions on fish and seafood consumption (FDA and EPA, 2004), nutritionists and physicians recommend large intake of these foods due to important nutritional properties, such as the Omega-3 (Lee *et al.* 2009). Practically all species of fish show some contamination by environmental pollutants, to a greater or lesser degree. Consequently, the higher the consumption of these foods, the higher the individual exposure to MeHg and other pollutants such as PCBs (Oken *et al.* 2012). In this context, studies investigating possible effects of chemical mixtures on different systems and organs, using relevant doses, are necessary.

MeHg is an organic form of mercury, converted by the action of aquatic microorganisms (NRC, 2000). Experimental and epidemiological studies have shown that MeHg is associated with neurotoxic effects, motor impairment and damage in kidneys, immune and cardiovascular system, besides a possible carcinogen role to humans (Grotto *et al.* 2011). Other studies have reported adverse effects of MeHg on reproductive parameters in humans and experimental animals, including impairment of libido, erectile dysfunction, hypospermia, apoptosis of germ cells leading to testicular atrophy, low serum testosterone levels and infertility. Nevertheless, its potential adverse effect on the synthesis and metabolism of steroids still requires further investigation (Fossato da Silva *et al.* 2011).

Together with MeHg, PCBs are also important contaminants of fish, produced largely during the years 1970 to 1980. Their resistance to degradation and their high dispersion across oceans and air pollutants has made them important agents right up to the present day (Lignell *et al.* 2009; Polder *et al.* 2009). Human exposure usually occurs at low doses and for long periods (Haave *et al.* 2011). Even low levels of PCBs, such as those often found in some human tissues and breast milk, are capable of causing adverse effects on reproductive parameters, development and endocrine function (Emmet *et al.* 1988). In experimental animals, exposure to 100 mg/kg of PCB 153, an abundant type of PCB, induced apoptosis of Leydig cells responsible for steroidogenesis in males (Oskam *et al.* 2004). PCBs are produced commercially as Aroclor, a mixture of them (Strużyńska *et al.*, 2012). The administration of this mixture, under experimental conditions, also results in decreased levels of thyroid hormones, even in low doses, and causes significant reduction in sperm reserves in the cauda epididymis when administrated at the dose of 25 mg/kg (Gray *et al.* 1993).

Despite the known need to investigate MeHg exposure associated with other pollutants such as PCBs, the number of studies addressing this problem is limited. In addition, special attention is given to neurobehavioral endpoints and exposure during adulthood or prenatal and lactation life. In this context, there is a scarcity of studies investigating reproductive aspects after exposure to MeHg and PCBs associated, especially in models of exposure during pre- and peripuberty. Puberty is a complex biological process regulated by the endocrine system, but the factors involved in this modulation are still poorly understood (Maranghi & Mantovani 2012; Perobelli 2014). Exposure to chemicals during childhood deserves special attention, because at this stage the individual is, for the first time, in contact with environmental contaminants without the mediation of maternal metabolism as occurs during pregnancy and lactation. Moreover, the periods of pre- and peri-puberty are considered critical stages of male sexual development, as they are periods of rapid and interactive changes in endocrine and morphologic aspects (Stoker *et al.* 2000). This study aimed to investigate whether prepubertal exposure to fish contaminants MeHg and PCBs associated at low doses causes immediate and late injuries on histology and morphometry of reproductive organs, liver and kidney of male rats.

Material and methods

Animals

To ensure the origin and age of animals used in the experiment, 23 pregnant female Wistar rats were obtained from the *biotherium* of the 'Universidade Sagrado Coração'. On the day after natural birth of litters (PND 1), the gender of pups was defined by comparing the anogenital distance. After that, it was standardized eight pups per dam, ensuring balanced nutrition for all the 23 litters. At the end of lactation period (PND 21), male offspring was separated.

During all the experiment, animals were allocated in polypropylene cages (one dam per cage during gestation and lactation and three male pups per cage after lactation), with laboratory-grade pine shavings as bedding. Rats were maintained under controlled temperature (±23°C) and lighting conditions (12L/12D photoperiod). Rat chow and filtered tap water were provided *ad libitum*.

Ethical approval statement

Experimental procedures were in accordance with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation and were approved by the 'Universidade Sagrado Coração' Ethics Committee for Animal Research (protocol number 30/13).

Experimental design

Male rats on PND 21 were distributed randomly into 5 groups, as specified in Table 1. Rats from the same litter

were separated in different experimental groups. The doses of MeHg (Sigma-Aldrich, Brasil, (São Paulo/SP, Brazil), purity 99.9%) used in this study are considered low doses and were based on previous studies (Passos *et al.* 2008; Grotto *et al.* 2011; Guo *et al.* 2012). The doses selection of Aroclor 1254 (Sigma-Aldrich) was based on the NOAEL of this compound (Gray *et al.* 1993). MeHg was diluted in distilled water and Aroclor in corn oil (volume 1 ml/kg). Treatment of the animals was conducted from PND 23 to 53, by gavage, according to the protocol suggested by the Environmental Protection Agency of the United States (USEPA, 2011), to study juvenile toxicity. Animals were weighed and treated daily for 30 days, always in the morning.

Evaluation of preputial separation

The age at acquisition of preputial separation, indicator of puberty onset, was evaluated daily, starting on PND 30, by manual retraction of prepuce (Parker 2006).

Body weight of animals

During treatment, all animals were weighed daily, before receiving their respective treatment by gavage, to adjust the toxicant daily dose to body weight. The end of treatment period and the euthanasia of part of the animals occurred on PND 53. The remaining animals were kept until PND 115 and weighed every two days. These animals were euthanized after 62 days without exposure to MeHg and Aroclor between PND 53 and PND 115. This period corresponds to a complete spermatogenesis, whose duration varies from 52 to 53.2 days depending on the strain of rat (Clermont 1972), and a complete sperm transit through the epididymis, which has approximately eight-day duration in rats (Amann *et al.* 1976;. França *et al.* 2005.). The aim of this second euthanasia was to evaluate late effects and the capacity of recovery of the parameters evaluated.

Euthanasia procedures and endpoints evaluated

On PND 53 (immediate evaluation, $n = 8$ rats/experimental group) or PND 115 (late evaluation, $n = 9$ rats/experimental group), the animals were euthanized for the evaluation of organ weights and histomorphometric parameters of the testes, epididymis, liver and kidneys. Rats were anaesthetized using a combination of xylazine with ketamine, and then decapitated after this procedure. The right testis and epididymis, prostate, full seminal vesicle, vas deferens, liver, kidneys and adrenals were removed and their wet weights (absolute and relative to body weight) were recorded. Liver and kidneys were fixed in a solution of 10% buffered formalin for 24 h. Right testis and epididymis were fixed in Bouin's fluid for 24 h (30% formaldehyde, 70% saturated picric acid, 5% glacial acetic acid) and fixed in Bouin's fluid for 24 h (25% formaldehyde, 70% saturated solution of picric acid and 5% glacial acetic acid) respectively. The pieces were embedded in paraffin wax and sectioned at 5 μm (cross sections of the testis and longitudinal sections of the epididymis). Sections used for histological evaluation were stained with haematoxylin and eosin (HE), examined and photographed by light microscopy.

Table 1 Experimental design

Experimental groups	
Group 1 (G1, $n = 17$)	Control. Received only the vehicle (corn oil)
Group 2 (G2, $n = 17$)	MeHg. Received only the MeHg at dose of 0.5 mg/kg/day
Group 3 (G3, $n = 17$)	Aroclor. Received only the Aroclor at dose of 1 mg/kg/day
Group 4 (G4, $n = 17$)	Low mix: MeHg (0.05 mg/kg/day) + Aroclor (0.1 mg/kg/day)
Group 5 (G5, $n = 17$)	High mix: MeHg (0.5 mg/kg/day) + Aroclor (1.0 mg/kg/day)

Histopathology evaluation

Testis. Seminiferous tubules were evaluated quantitatively. Cross sections were randomly chosen in three non-serial sections per animal, totalling 100 tubules/animal. Seminiferous tubules sections were classified as normal (presence of concentric and normally organized germ cell layers in seminiferous epithelium) or abnormal (presence of germ cells and cellular debris in the lumen, multinucleated formation, few germ cell layers, vacuole formation, occurrence of sloughed nuclei in the tubular lumen, Sertoli cell nucleus displacement). Testicular interstitial connective tissue was evaluated in a qualitative method.

Epididymis, liver and kidneys. The histopathological analysis was performed qualitatively according to the criteria published by The Society of Toxicologic Pathology (SSNDC, 2014) and was performed in blind assays. The photomicrographs for documentation were captured by a digital camera (QImaging Micropublisher 3.3 Cooled; QImaging and Photometrics, Surrey, British Columbia, Canada) coupled to the microscope (Nikon Eclipse 80i; Nikon Instruments Inc., N.Y., USA).

Morphometric evaluation

Degree of maturation of the seminiferous epithelium (only in 53-day-old animals). To evaluate maturation degree of the seminiferous epithelium, 100 cross sections of seminiferous tubules per animal were evaluated randomly, using the adapted method of assigning values according to the type of mature germ cell most numerous in tubular epithelium (Lamano-Carvalho *et al.* 1996): degree 1 – spermatocytes I or II; degree 2 – young spermatids with rounded nucleus (stages 1–8 of spermiogenesis); degree 3 – spermatids in maturation phase, with ovoid or elongated nucleus (stages 9–14 of spermiogenesis); degree 4 – spermatids in maturation phase, with elongated nucleus (stages 15–18 of spermiogenesis);

degree 5 – mature spermatids (stage 19 of spermiogenesis) in small quantity; degree 6 – mature spermatids (stage 19 of spermiogenesis) in average amount; degree 7 – mature spermatids (stage 19 of spermiogenesis) in larger amount. The number of seminiferous tubules in each degree was multiplied by its degree, and then the values were added and divided by 100, resulting in the 'average degree'.

Seminiferous tubule diameter and height of the seminiferous epithelium. Diameters of seminiferous tubules and thickness of seminiferous epithelium were determined in 20 sections of seminiferous tubules per animal, at stage 9 of spermatogenesis (Leblond & Clermont 1952).

Sertoli cell number. Sertoli cells' nuclei were counted in 20 cross sections of seminiferous tubules per rat at stage 9 of spermatogenesis classified according to Leblond and Clermont (1952).

Statistical analyses

To compare quantitative data, statistical tests were used: analysis of variance (ANOVA) test with *a posteriori* Dunnett or nonparametric Kruskal–Wallis test with *a posteriori* Dunn, according to the characteristic of each variable. Differences were considered statistically significant when $P < 0.05$.

Results

Body weight gain was similar among experimental groups during treatment as well as after this period (Figure 1a and b respectively). The same was observed in the age of preputial separation (Figure 1c). At both studied ages (PND 53 and 115), final body weight and absolute and relative wet weights of organs (testis, epididymis, prostate, full seminal vesicle, vas deferens, kidneys, liver, adrenals and thyroid) were similar among the five experimental groups (data not shown).

Histological analysis of seminiferous tubules (Table 2, Figure 2) and epididymidis (data not shown) did not reveal alterations in treated groups compared with the control group, in both studied ages. However, significant morphologic changes were observed in the testicular interstitium of all treated groups at adulthood (PND 115), compared with control animals. The testicular interstitial connective tissue was expanded due to interstitial oedema (Figure 2, arrows and asterisks). In addition, testicular morphometry

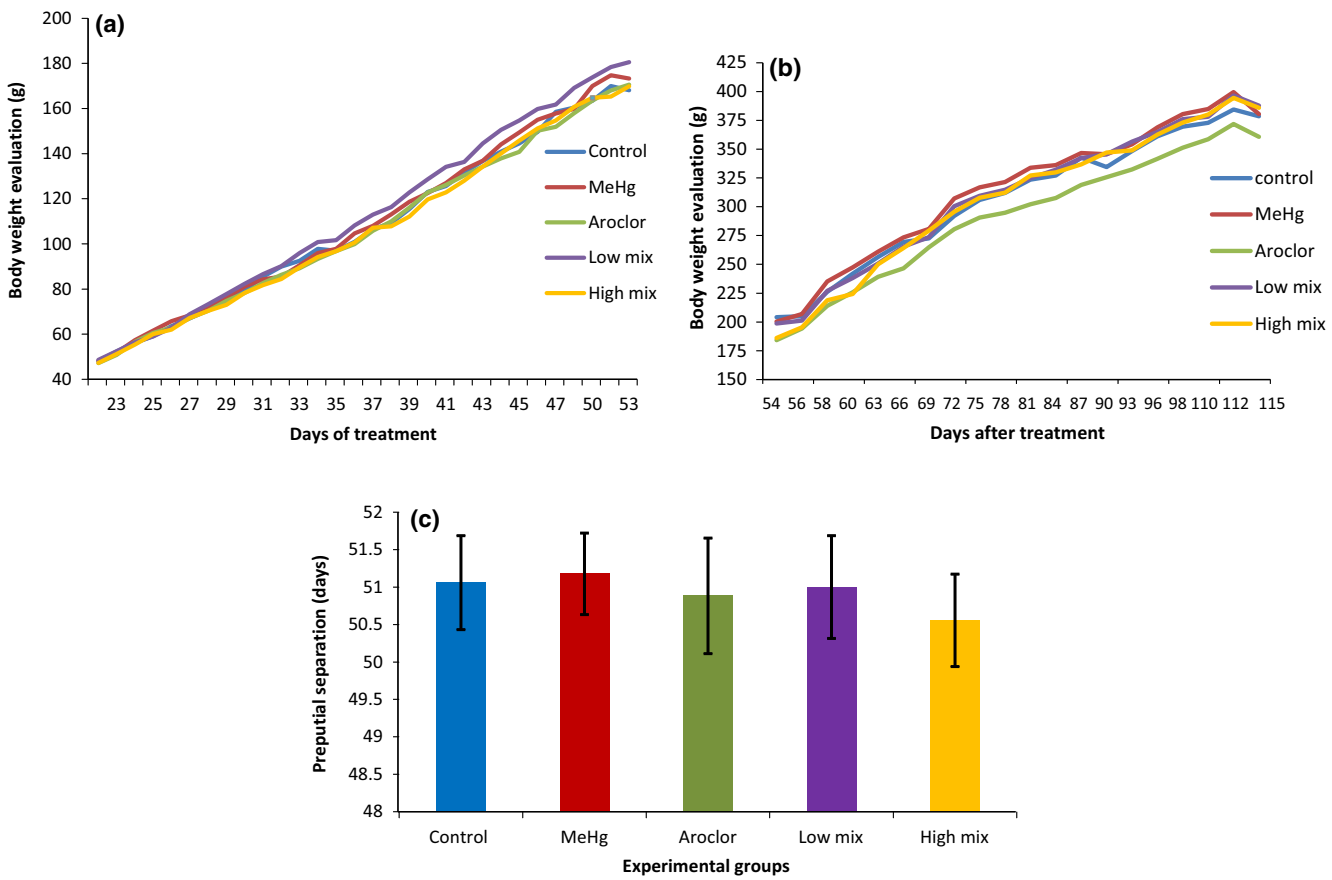

Figure 1 (a) Body weight evaluation of animals during treatment period (30 days), from PND 23 to 53, $n = 17$ animals/group; (b) body weight evaluation of animals during recovery period (62 days), from PND 54 to 115, $n = 9$ animals/group; (c) Average age of preputial separation of the animals, $n = 17$ animals/group. Values expressed as mean ± SEM. ANOVA with *a posteriori* test of Dunnett. PND, postnatal day.

parameters showed several changes in the pattern of male gonad organization at peripuberty and adulthood (Table 2). There was a delay in the degree of seminiferous epithelium maturation in all treated groups in comparison with the control group. On the other hand, only the animals from Aroclor group showed a decrease in seminiferous epithelium height, seminiferous tubules diameter and Sertoli cell number when compared to the control group on PND 53. At adulthood, all treated groups showed an increase in seminiferous epithelium height and seminiferous tubule diameter in comparison with the control group. In addition, the experimental animals from Aroclor group as well as those of low mix and high mix groups showed a reduction in number of Sertoli cells (Table 2).

In both ages studied hepatic parenchyma retained a normal aspect in all experimental groups, without any significant alteration such as degeneration, necrosis, fibrosis or inflammation (data not shown). However, histological alterations were observed in the kidneys of chemical-exposed animals, on PND 53 and 115. Animals from MeHg group

Table 2 Immediate (PND 53) and late (PND 115) evaluation of testes' histopathology and morphometry of experimental rats

	Control	MeHg	Aroclor	Low mix	High mix
53 days old					
Histopathology					
Normal ST (%)	91.37 ± 1.28	92.37 ± 1.12	92.50 ± 1.15	90.75 ± 2.13	93.12 ± 1.33
Abnormal ST (%)	8.62 ± 1.28	7.62 ± 1.12	7.50 ± 1.15	9.25 ± 2.13	6.87 ± 1.33
Morphometry					
Maturation degree of SE	4.16 ± 0.13	3.63 ± 0.09*	3.65 ± 0.09*	3.37 ± 0.09*	3.55 ± 0.09*
ST diameter (μm)	253.52 ± 1.60	250.95 ± 1.56	242.42 ± 2.34*	258.20 ± 1.66	253.08 ± 1.62
SE height (μm)	84.88 ± 0.72	83.56 ± 0.67	79.40 ± 0.89*	85.29 ± 0.74	82.27 ± 0.68
Sertoli cell number	18.26 ± 0.60	16.85 ± 0.42	15.78 ± 0.89*	18.75 ± 0.50	17.55 ± 0.44
115 days old					
Histopathology					
Normal ST (%)	93.44 ± 1.16	90.62 ± 0.80	90.66 ± 1.40	90.90 ± 0.85	91.60 ± 1.24
Abnormal ST(%)	6.55 ± 1.16	9.37 ± 0.80	9.33 ± 1.40	9.10 ± 0.85	8.40 ± 1.24
Morphometry					
ST diameter (μm)	258.25 ± 1.76	267.36 ± 1.53*	279.74 ± 1.87*	271.41 ± 1.56*	274.05 ± 1.65*
SE height (μm)	81.22 ± 0.67	87.93 ± 1.02*	88.40 ± 0.86*	84.75 ± 0.57*	85.72 ± 0.66*
Sertoli cell nuclei	19.70 ± 0.47	18.52 ± 0.77	16.98 ± 0.28*	14.91 ± 0.31*	15.61 ± 0.44*

Values expressed as mean ± SEM. ANOVA with *a posteriori* test Bonferroni. Control (n = 8–9), MeHg (n = 8–9), Aroclor (n = 8–9), low mix (n = 8–9) and high mix (n = 8–9). *Indicates statistical significance ($P < 0.05$) of chemical-exposed groups in relation to control. ST, seminiferous tubules; SE, seminiferous epithelium; PND, postnatal day.

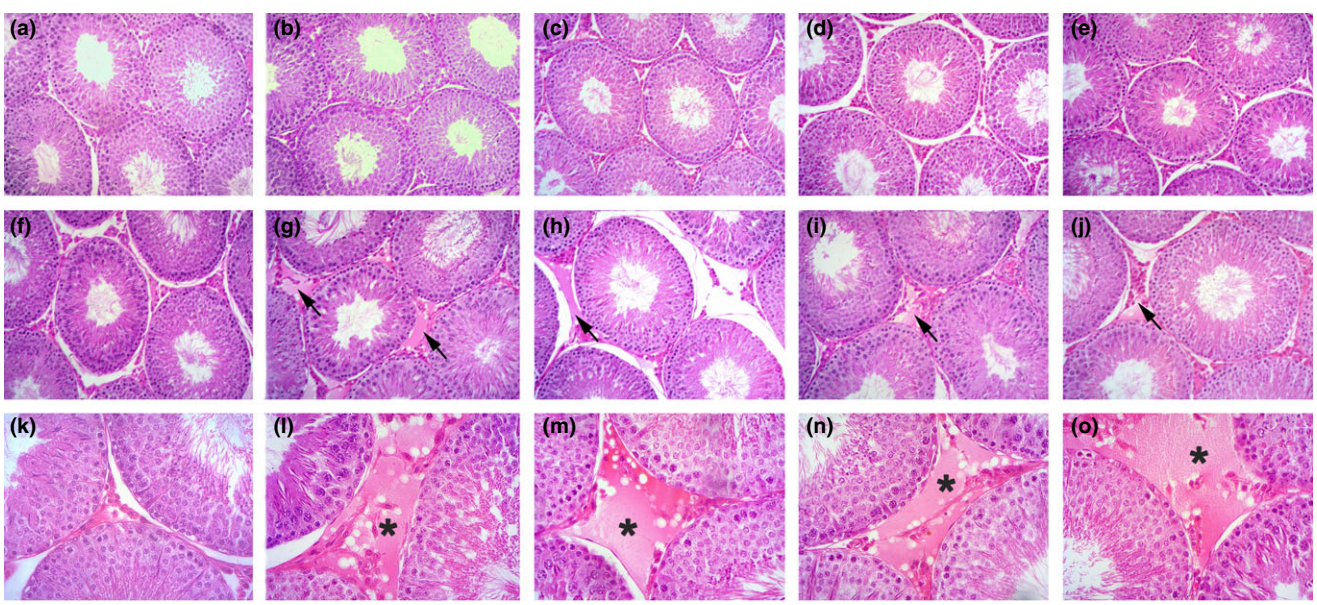

Figure 2 Photomicrographs of seminiferous tubules cross sections of animals on PND 53 (a–e) and on PND 115 (f–j). 200× final magnification. Detail of testicular interstitium oedema on photomicrographs (k–o). a, f, k (400× final magnification, asterisks). Control (n = 8–9); b, g, l: MeHg (n = 8–9); c, h, m: Aroclor (n = 8–9); d, i, n: low mix (n = 8–9); e, j, o: high mix (n = 8–9).

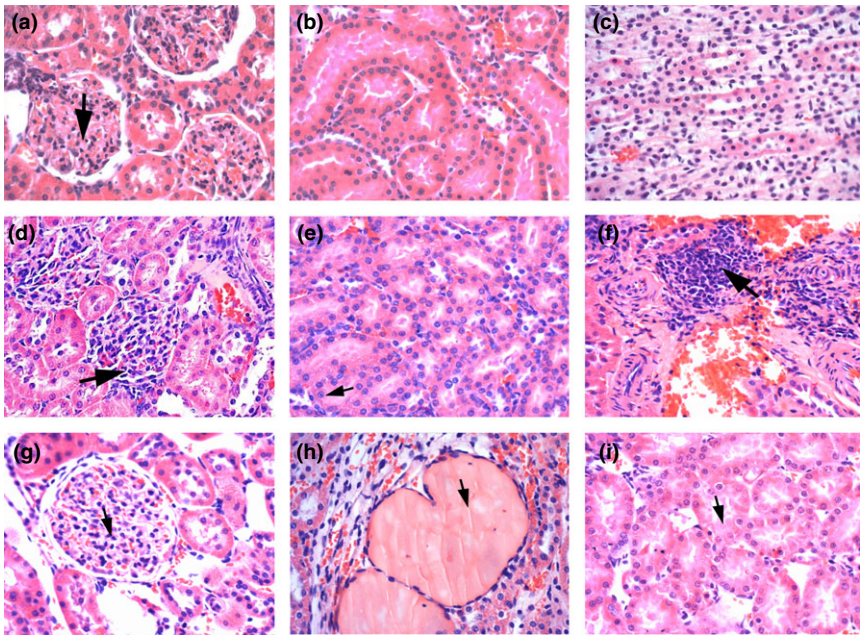

Figure 3 Photomicrographs of renal longitudinal sections of animals on PND 53 (a–e) and PND 115 (f–i). a: control, renal glomerulus in cortical region; b: control, renal tubules in cortical region; c: control, medullary region; d: MeHg, glomerular hypercellularity; e: Aroclor, degeneration of renal tubules; f: MeHg, inflammatory infiltrate; g: Aroclor, glomerular hypercellularity; h: low mix, hypotrophy of tubular cells; i: high mix, degeneration of renal tubules. Final magnification 400×; n = 8–9 animals/group.

showed glomerular hypercellularity at the age of 53 days and inflammatory infiltrate at the age of 115 days. Aroclor group showed renal tubular degeneration at the age of 53 days and glomerular hypercellularity at the age of 115 days, while hypotrophy of tubular cells and degeneration of renal tubules were observed in low mix and high mix groups at adulthood respectively (Figure 3).

Discussion

Methylmercury (MeHg) and polychlorinated bisphenols (PCBs, commercial mixture Aroclor) are persistent toxicants present mainly in seafood, suffering bioaccumulation in food chain. Despite the known need to investigate MeHg exposure associated with other pollutants such as PCBs, this approach is still quite uncommon and, consequently, the literature is limited. This context is even more critical considering studies that focus on individuals exposed to chemicals during their juvenile phase, that is the period of pre- and peri-puberty. Concerning this issue, the present study investigated the juvenile reproductive toxicology of MeHg and Aroclor, a mixture of fish contaminants, at low doses.

In the present study, absence of changes in body weight gain during the treatment period, as well as after it, indicates that chemical exposure did not induce systemic toxicity. Thus, body weight gain was not a contributing factor in any reproductive effects observed after prepubertal chemical exposure. Changes in final body weight or in the pattern of body weight gain during experimental period could reflect adverse responses of the animal to experimental conditions, such as treatment-induced anorexia, systemic toxicity, among others (EPA, 1996; Clegg *et al.* 2001). Also according to Clegg *et al.* (2001), absolute and relative weights of reproductive organs can be used for evaluating toxic effects of a substance on male reproductive system. These parameters were similar among all experimental groups, in immediate (PND 53) and late (PND 115) evaluation. As reported by Ashby and Lefevre (2000), changes in reproductive organ weights and on the day of preputial separation are the two main parameters for detecting anti-androgens in peripubertal male rat assays, as recently reaffirmed by Perobelli *et al.* (2012). Thus, the absence of adverse effects in these parameters could indicate no androgenic or anti-androgenic activity of the tested toxicants, isolated or in mixture.

In the same way, no adverse effects were observed in histopathology of seminiferous epithelium, epididymis and liver of treated rats in both studied ages. These data are different from those of Frenedoso da Silva *et al.* (2014) who observed histological alterations in testes of mouse exposed to MeHg (140 μg/kg, during 100 days) and from those of Pal and Ghosh (2012) who reported disorganization of cell plates and sinusoidal congestion in liver of rats treated with MeHg at 5 mg/kg for 15 days. These differences occurred probably due to the peculiarities of experimental design adopted in our study, including juvenile male rats together with the use of low doses. On the other hand, we observed an oedematous interstitium in testes of treated rats at adulthood, indicating a late effect of the chemical exposure, as

this alteration was not observed in immediate evaluation (on PND 53). Testicular fluid is distributed into two compartments: the seminiferous tubules and the interstitium. Seminiferous tubule fluid is synthetized by the Sertoli cells, and efferent ductules regulate its volume through reabsorption. Interstitial fluid originates primarily from the testicular vasculature. Several studies have indicated that the levels of interstitial fluid can be influenced by different factors, including testosterone, LH, GnRH agonists, Leydig cell factors other than testosterone, and factors produced by Sertoli cells (Porter *et al.* 2006). In the present study, this testicular abnormality could be related especially to Sertoli cell factors, as the androgen-dependent parameters were unaffected by the treatment (preputial separation and reproductive organs weight) and the number of these cells decreased in the animals from Aroclor, low mix and high mix groups.

Morphometry of testes showed that the degree of maturation of seminiferous epithelium was delayed in all treated groups in relation to the control group, suggesting an arrest in the onset of spermatogenesis process. In addition, at adulthood, all treated groups showed an increase in seminiferous tubules diameter and epithelium height. With these results, we hypothesized that Sertoli cell may be a target of this treatment in juvenile animals, especially of Aroclor exposure, inducing alteration in testicular organization and possibly in spermatogenesis process in the long term. In fact, a preview study showed that Aroclor 1254 is able to disrupt adult Sertoli cell metabolic functions *in vitro* (Krishnamoorthy *et al.* 2005), but there are no data regarding juvenile Sertoli cells. It is important to emphasize that morphometric results are considered late effects of the treatment, as immediate evaluation showed this result only in animals from Aroclor group. Besides, the obtained data suggest that MeHg and Aroclor did not show a synergistic interaction on reproductive outcomes. This hypothesis corroborates Rignell-Hydbom *et al.* (2007), who did not observe any synergistic effects between MeHg and CB-153 in semen samples from Swedish fisherman.

Investigation of renal histology of experimental animals revealed that the exposure to MeHg caused, mainly, immediate effect (observed at the end of treatment period, on PND 53). On the other hand, Aroclor induced permanent lesions, observed even after the 62-day interval period. So, further investigation on toxicokinetics of Aroclor is required to clarify this late effect. The urinary tract is not the principal route of excretion of MeHg. Only 10% of the total MeHg is excreted by the urinary tract (Hong *et al.* 2012) as MeHg undergoes demethylation and is excreted in the faeces (Abernethy *et al.* 2010). After the 62-day interval, degeneration produced by MeHg was totally reversed, although still has been observed an discrete mononuclear infiltration accompanied by glomerular hypercellularity. In Aroclor group occurred a predominance of moderate mononuclear infiltrate in all samples. In low mix and high mix groups, histopathological analysis revealed an intense mononuclear infiltrate, but there were no tubular degeneration signals. Treatment with Aroclor produced a late effect, showing tubular degeneration and interstitial infiltration by mononuclear cells. Treatments with MeHg and Aroclor in mixture did not cause tubular degeneration, but induced mononuclear infiltration, with the predominance of lymphocytes and glomerular hypercellularity. In one of these animals, it was observed hyaline casts. Pathak and Kundu (2013) demonstrated that the renal toxicity attributed to Aroclor is not dose dependent, but the length of exposure is crucial for the production of such adverse effects.

Conclusion

Fish contaminants delayed the establishment of spermatogenesis process in rat testis and affected histomorphometric organization of male gonads in the experimental conditions adopted in this study, together with injuries on renal tissue. A probable target of the treatment in reproductive system was Sertoli cells, in which number of Sertoli was decreased and possible dysfunctions could justify the other testicular alterations observed. Curiously, the main deleterious effects were late outcomes, as they were present at adulthood, after a 62-day recovery period. Another important point is the absence of synergistic interaction between MeHg and Aroclor. Unfortunately, the absolute scarcity of studies on juvenile exposure to MeHg and PCBs did not provide any direct comparison between the obtained results and data available in the literature. As far as we know, this is the first study to elucidate the effects of MeHg and Aroclor in mixture using an experimental protocol of juvenile toxicity.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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